

Claims

1. A method for producing a protein of interest in a host cell, wherein said host cell has been genetically modified in order to express significantly reduced levels of a metalloprotease comprising a HXXEH motif (SEQ ID NO 1), compared to the corresponding
5 non-modified cell when cultured under identical conditions, the method comprising
 - a) introducing into the host cell a nucleic acid sequence encoding the protein of interest,
 - 10 b) cultivating the host cell of step (a) in a suitable growth medium for production of the protein of interest, and
 - c) isolating the protein of interest.
- 15 2. A method according to claim 1, wherein the metalloprotease further comprises a glutamic acid residue between 70 and 80 amino acids C-terminal of the second His residue in the HXXEH motif.
3. A method according to any of the preceeding claims, wherein the metalloprotease
20 further comprises a glycine residue 3 amino acids N-terminal of the first His residue in the HXXEH motif.
4. A method according to any of the preceeding claims, wherein the metalloprotease further comprises a glycine residue 5 amino acids C-terminal of the second His residue in
25 the HXXEH motif.
5. A method according to any of the preceeding claims, wherein the metalloprotease further comprises a lysine residue 8 amino acids C-terminal of the second His residue in the HXXEH motif.
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6. A method according to any of the preceeding claims, wherein the metalloprotease further comprises a tyrosine residue 9 amino acids C-terminal of the second His residue in the HXXEH motif.
- 35 7. A method according to any of the preceeding claims, wherein the metalloprotease further comprises a proline residue 10 amino acids C-terminal of the second His residue in the HXXEH motif.

8. A method according to any of the preceeding claims, wherein the metalloprotease further comprises the consensus sequence SEQ ID NO 2.
9. A method according to any of the preceeding claims wherein the metalloprotease further
5 comprises the consensus sequence SEQ ID NO 3.
10. A method according to any of the preceeding claims, wherein the metalloprotease further comprises a NAXTXXXXT motif between 20 and 30 amino acids C-terminal of the second His residue in the HXXEH motif.
- 10 11. A method according to any of the preceeding claims, wherein the metalloprotease is selected from:
- i) any one of the group consisting of SEQ ID NO's 4 to 15, and
15 ii) a sequence which is at least 80% identical to any one of SEQ ID NO's 4 to 15.
12. A method according to any of the preceeding claims, wherein the metalloprotease is at least 80% identical to the SEQ ID NO: 4.
- 20 13. A method according to any of the preceeding claims, wherein the total amount of the protein of interest is increased at least 5% compared the corresponding non-modified cell when cultured under identical conditions.
- 25 14. A method according to any of the preceeding claims, wherein the total amount of the protein of interest is increased at least 50% more than the corresponding non-modified cell when cultured under identical conditions.
15. The method according to any of the preceeding claims, in which the host cell is a
30 prokaryotic cell.
16. The method according to any of claims 1-14, in which the host cell is a eukaryotic cell.
17. The method according to claim 16, in which the host cell is a non-filamentous fungal
35 cell.
18. The method according to claim 16, in which the host cell is a filamentous fungal cell.
19. The method according to claim 17, in which the host cell is a strain of *Saccharomyces*.

20. The method according to claim 19, in which the host cell is *Saccharomyces cerevisiae*.
21. A host cell useful for the expression of a protein of interest, wherein said cell has been
5 genetically modified in order to express significantly reduced levels of a metalloprotease
comprising a HXXEH motif (SEQ ID NO 1) than the corresponding non-modified cell when
cultured under identical conditions.
22. A host cell according to claim 21, wherein the metalloprotease further comprises the
10 consensus sequence SEQ ID NO 3.